10090140.070902

45

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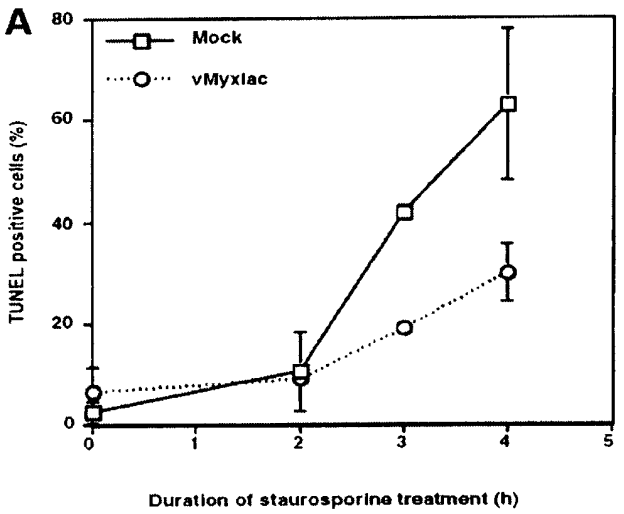
Figure 1



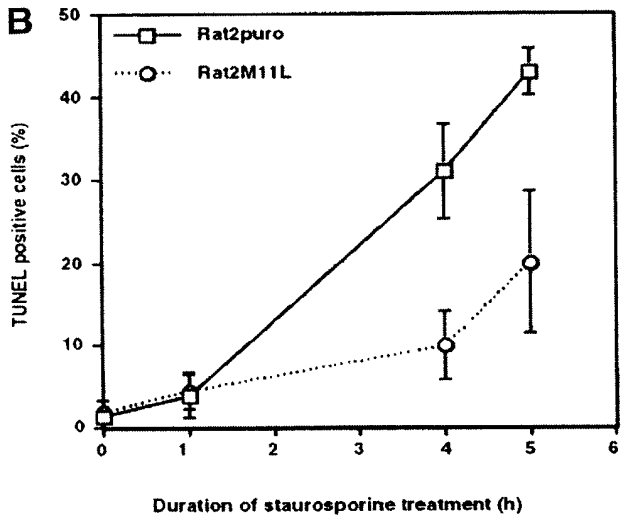


Figure 1 (con't)

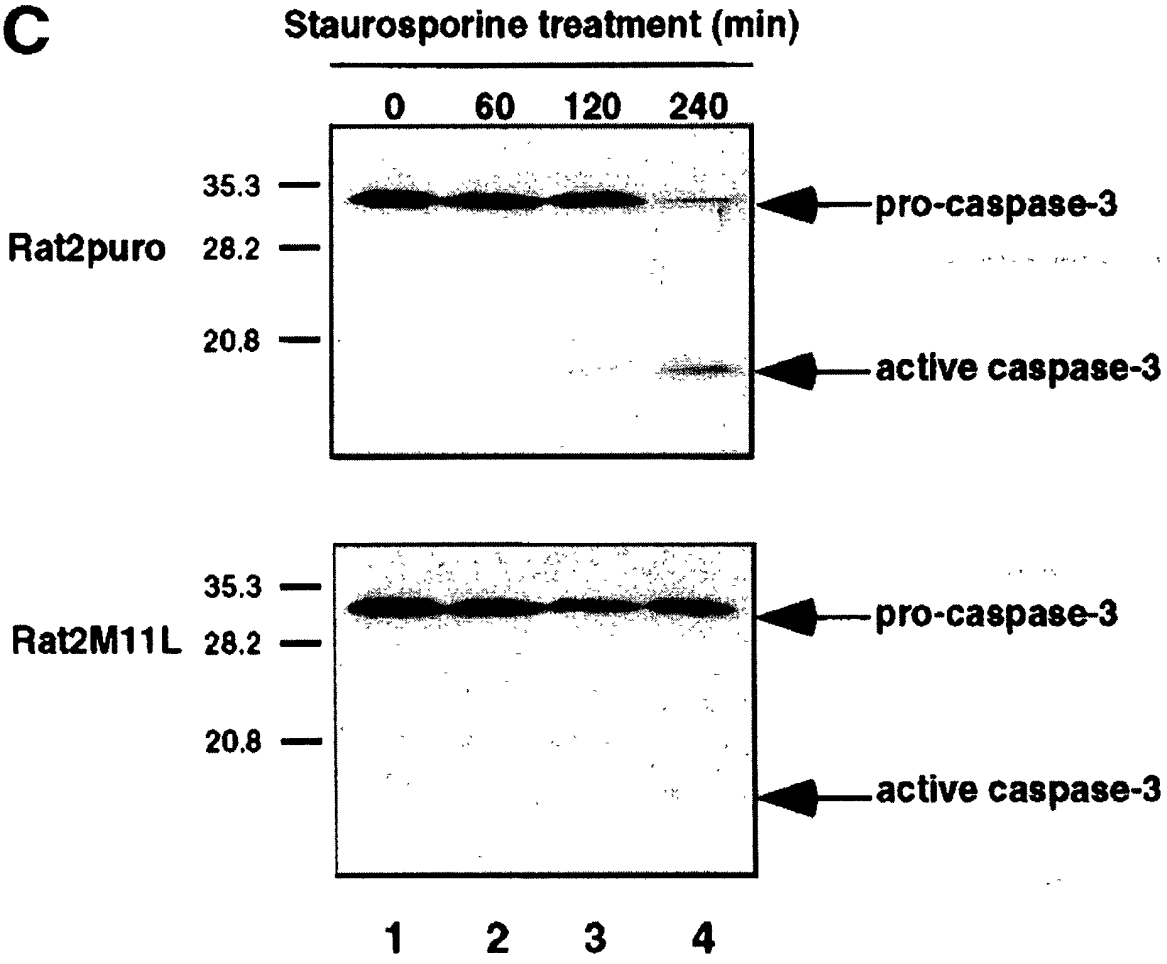


Figure 2

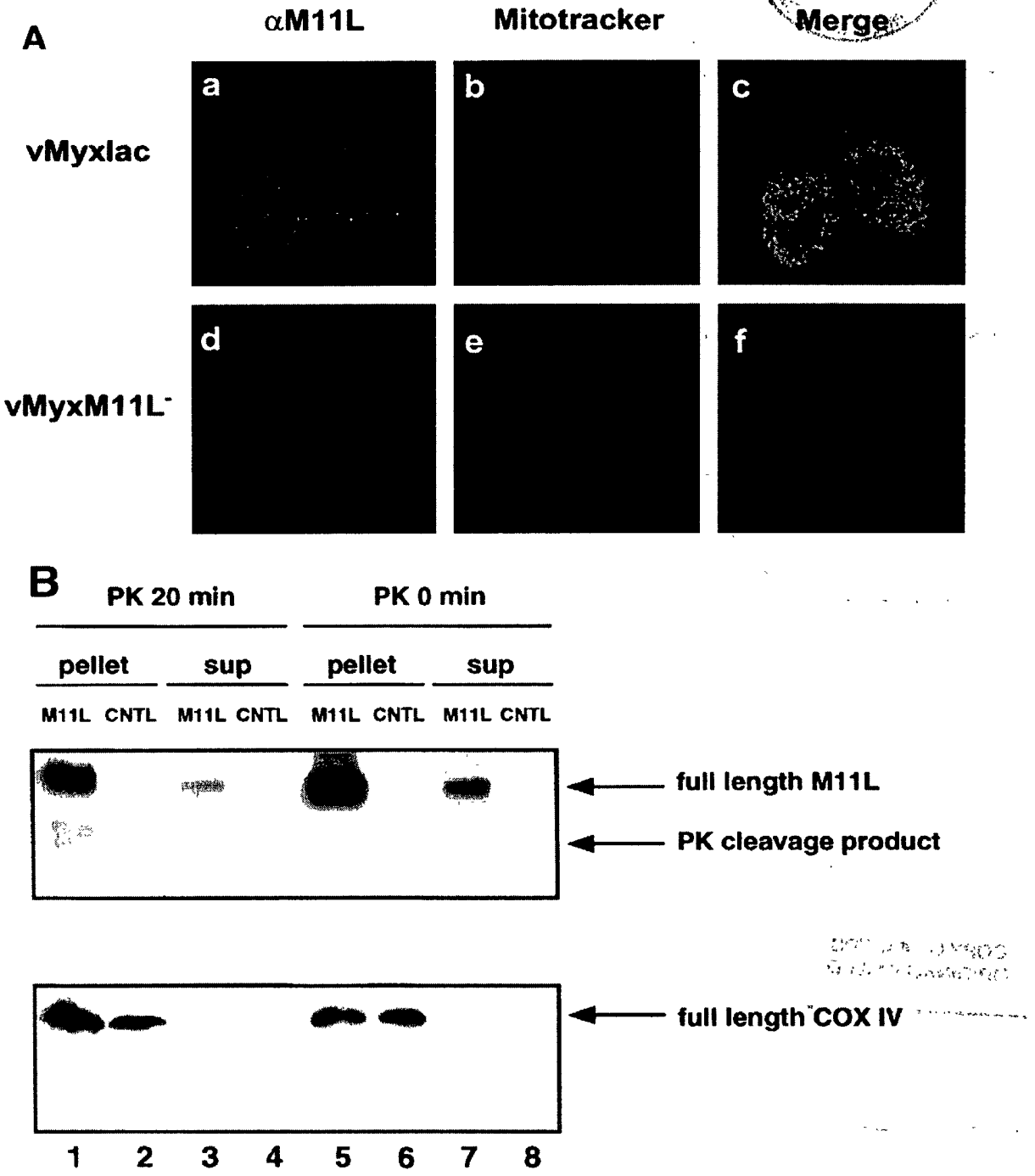

A
αM11L
Mitotracker
Merge
vMyxlac
vMyxM11L-
a
b
c
d
e
f
B
PK 20 min
PK 0 min
pellet
sup
pellet
sup
M11L CNTL M11L CNTL M11L CNTL M11L CNTL
full length M11L
PK cleavage product
full length COX IV
1 2 3 4 5 6 7 8

Figure 3

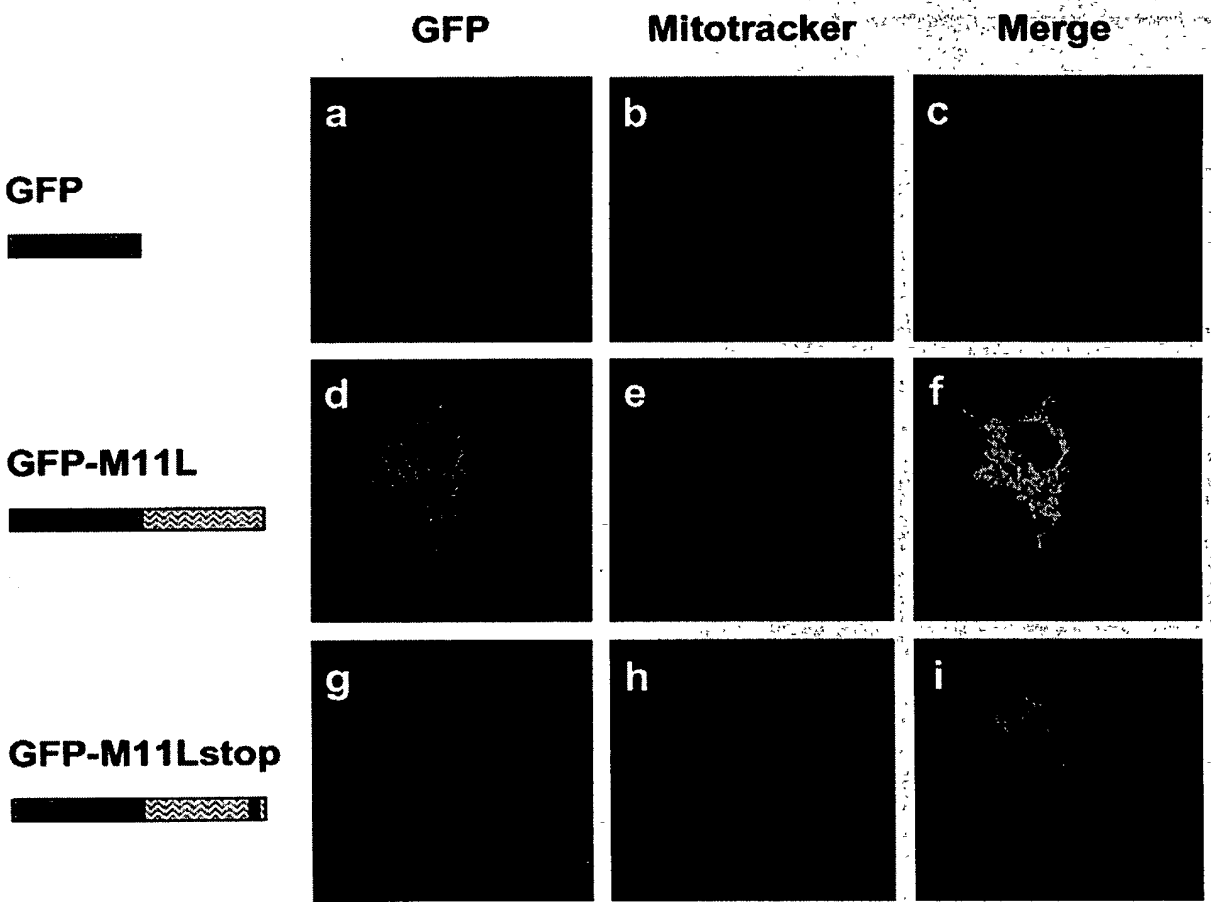
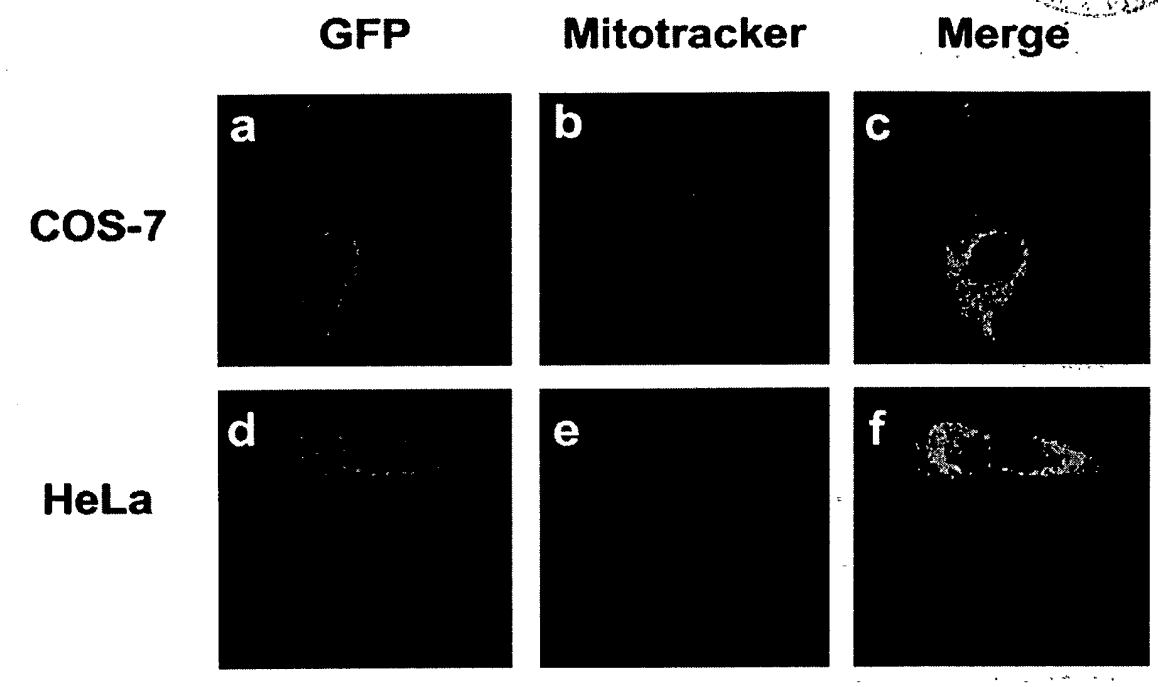
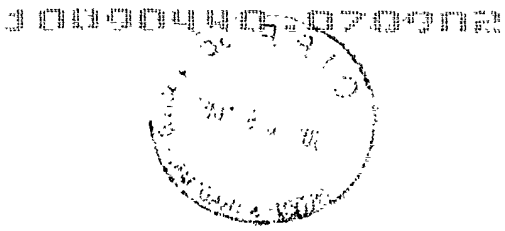


Figure 4


GFP
Mitotracker
Merge
a
b
c
COS-7
d
e
f
HeLa


GFP
mt
mt = KISVYLTAAVVGFVAYGILKWYRGT
+
+
+

Figure 5



					domain is a mitochondrial targeting signal	domain is required for function
M11L	K	ISVYLTAAVVGFVAYGIL	K	WYRGT	Y	Y
Bcl-2	K	TLLSLALVGACITLGAYLS	H	K	Y	Y/N
Bcl-X_L	R	WFLTGMTVAGVVLLGSLFS	R	K	Y	Y/N
Boo/Diva	R	LLIQAFLSGFFATAIFFIW	K	RL	?	?
CED-9	R	WSMIGAGVTAGAIGIVGVVVCG	R	MMFSLK	?	?
BHRF-1	R	FSWTLFLAGLTLSLLVICSYLFIS	R	GRH	Y	Y
KSbcl-2	R	MTALLGSIALLATILAAVAMS	R	R	?	?
Nip3	K	VFLPSLLLSHLLAIGLGIYIG	R	RLTTSTSTF	Y	Y
Nix	K	VFIPSLFLSHVLALGLGIYIG	K	RLSTPSA	Y	Y

positive charge — 18-24 aa putative membrane-spanning domain — positive charge — positive tail

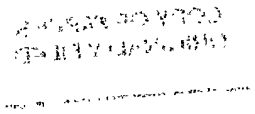


Figure 6

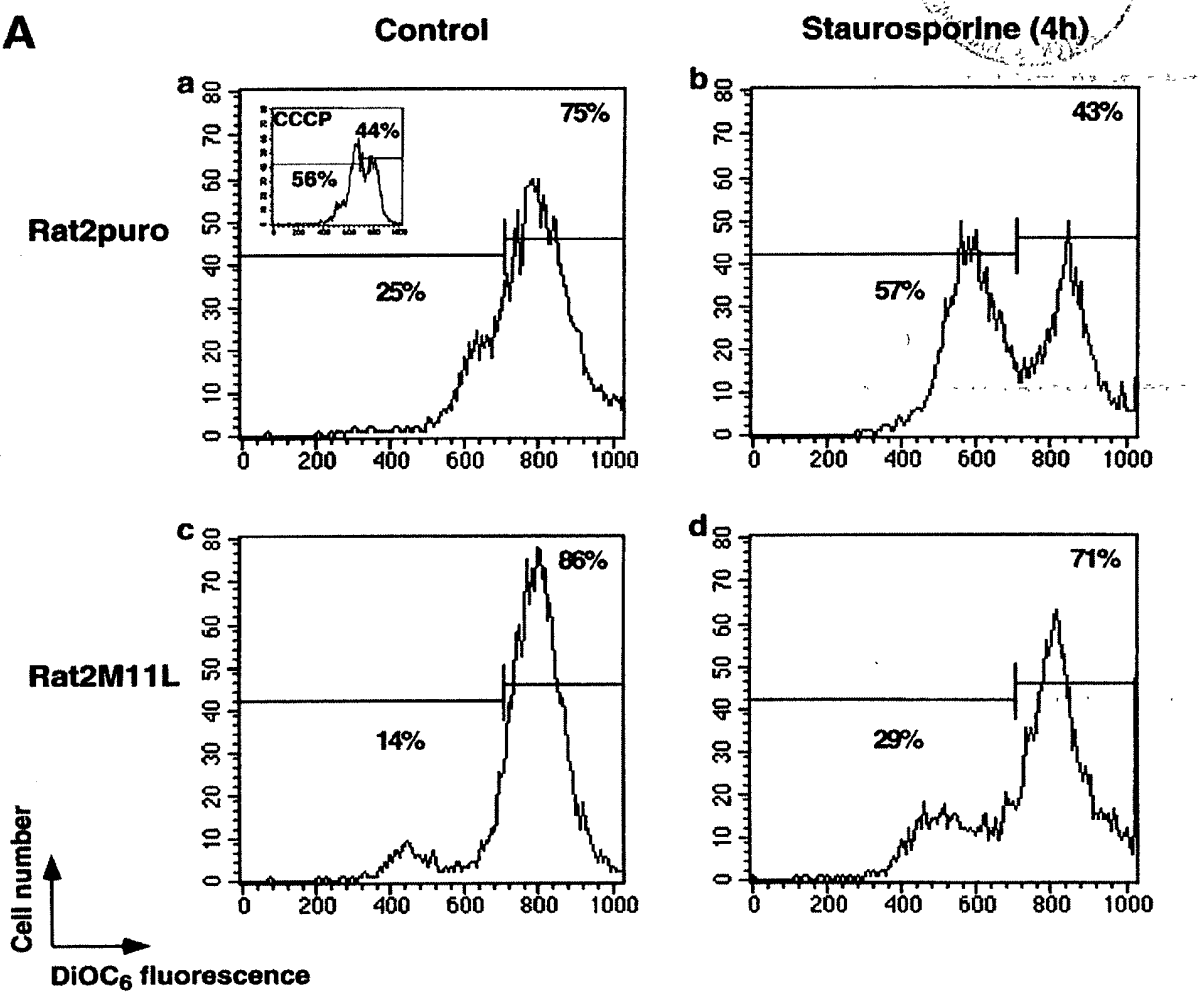

A
Control
Staurosporine (4h)
a
b
c
d
Rat2puro
Rat2M11L
CCCP
44%
56%
75%
25%
43%
57%
86%
14%
71%
29%
Cell number
DiOC6 fluorescence

Figure 6 (con't)

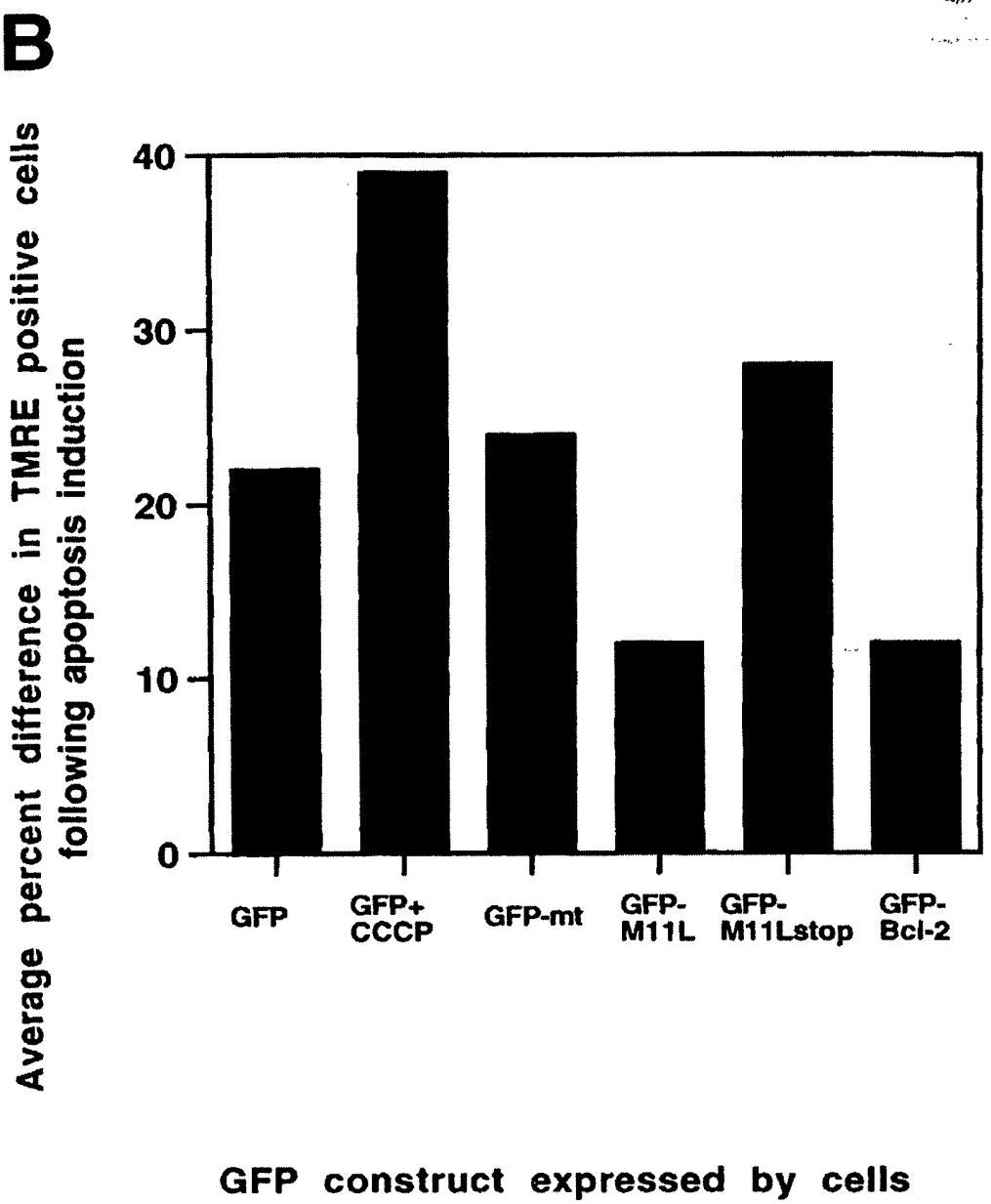
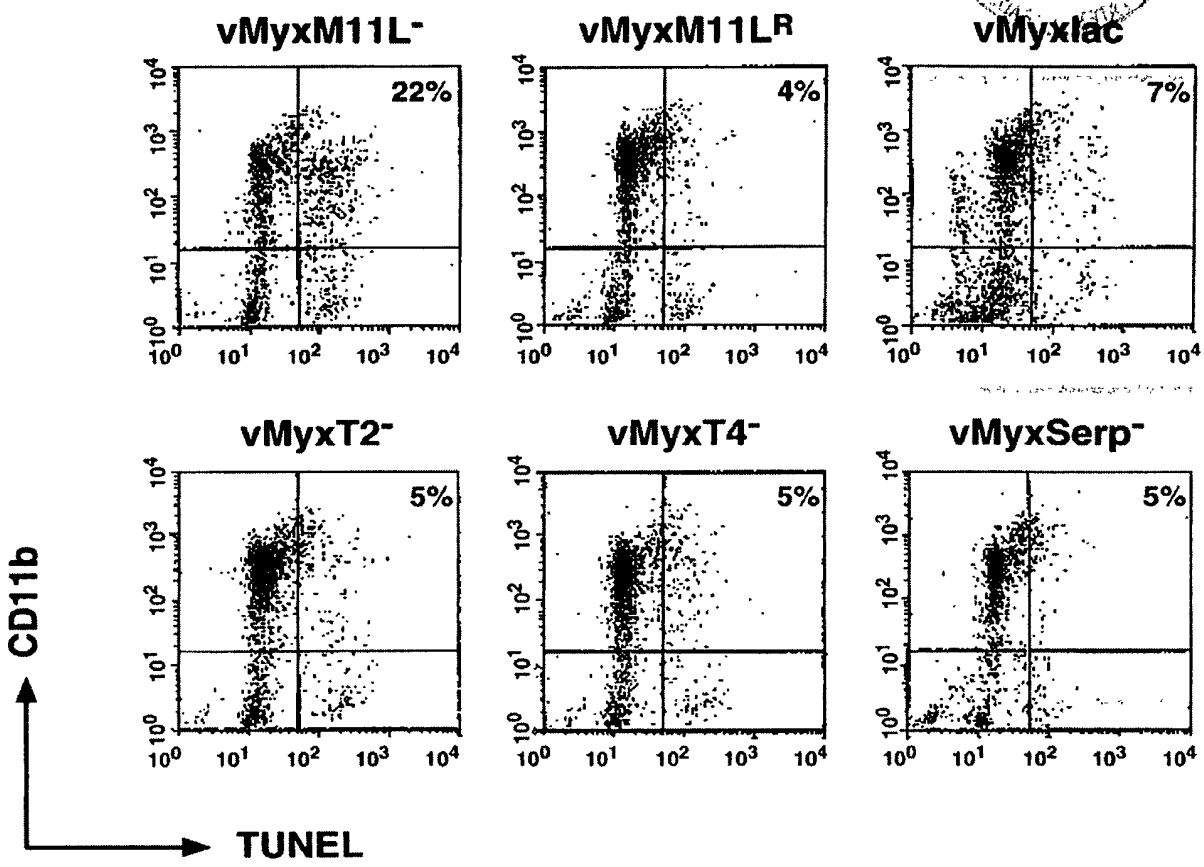


Figure 7


vMyxM11L-
vMyxM11LR
vMyxlac
22%
4%
7%
vMyxT2-
vMyxT4-
vMyxSerp-
5%
5%
5%
CD11b
TUNEL

Process to Identify M11L-interacting Proteins

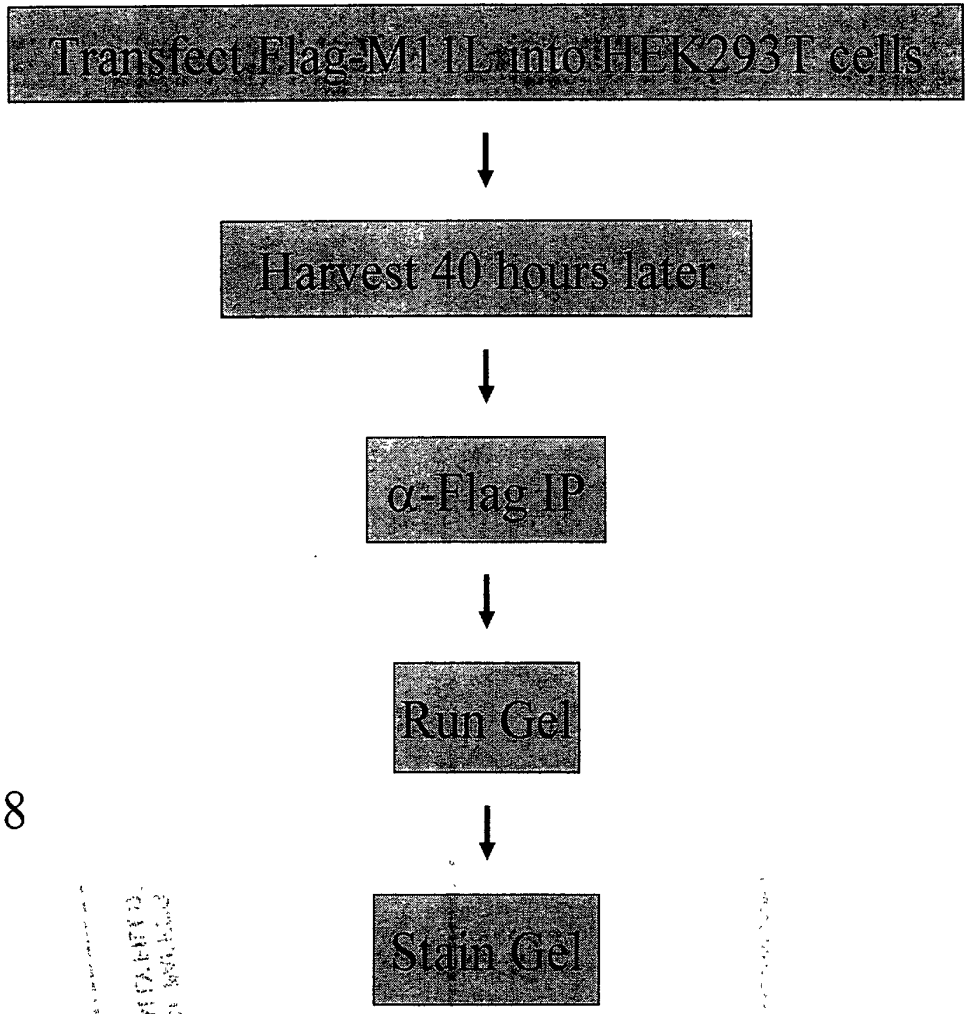
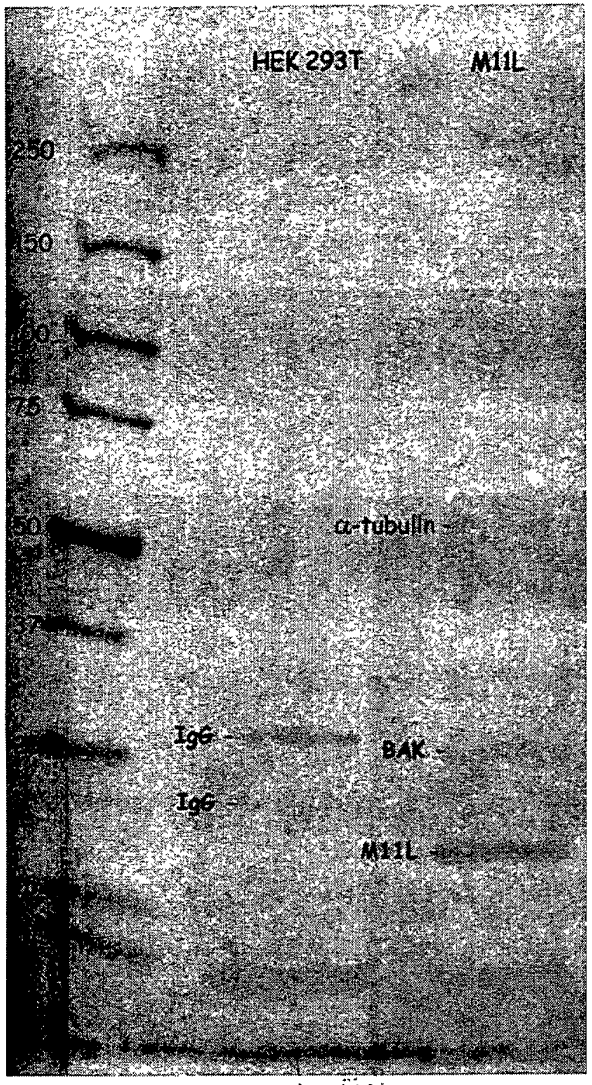


Figure 8


HEK 293T
M11L
250
150
100
75
50
37
α-tubulin
IgG
BAK
IgG
M11L

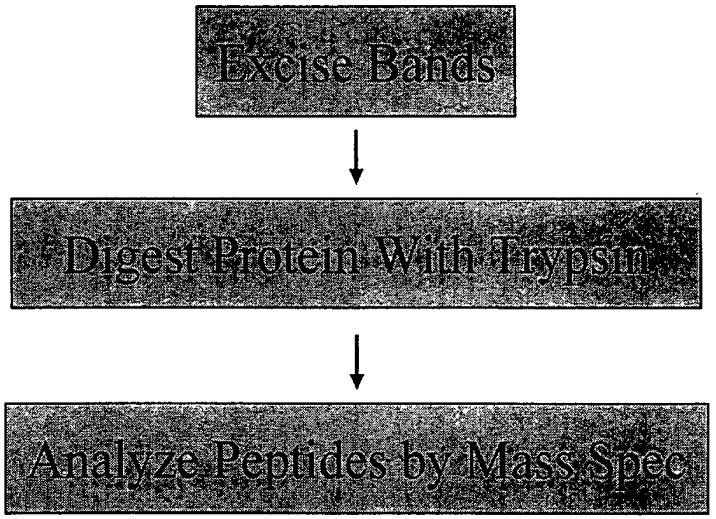

Excise Bands
Digest Protein With Trypsin
Analyze Peptides by Mass Spec

Figure 9

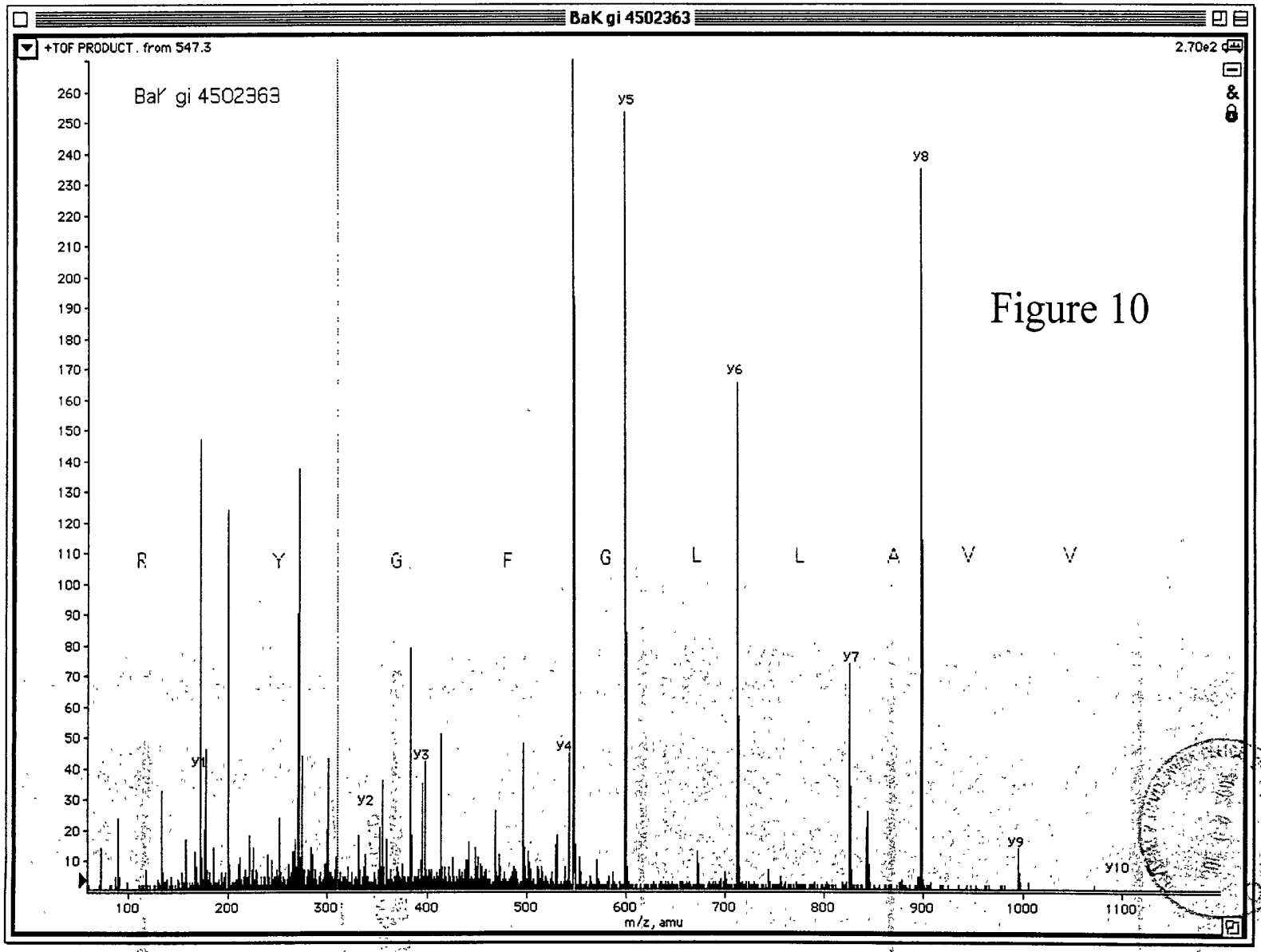

BaK gi 4502363
+TOF PRODUCT. from 547.3
2.70e2
BaK gi 4502363
R Y G F G L L A V V
y1
y2
y3
y4
y5
y6
y7
y8
y9
y10
m/z, amu

Figure 10

Bak, a Bcl2 family member, was identified by Mass Spec

MASGQGPGPPRQECGEPALPSASEEQVAQD

TEEVFRSYVFYRHQQEQEAEGVAAPADPEM

VTLPLQPSSTMGQVGR**QLAIIGDDINR**RYD

SEFQTMLQHLQPTAENAYEYFTK**IATSLFE**

SGINWGRVVALLGFGYRLALHVYQH

GLTGFLGQVTRFVVDFMLHHCIARWIAQRGGWVA

ALNLGNGPILNVLVVLGVVLLGQFVVRRFFKS

Figure 11